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Molecular characterization of a tomato polygalacturonase gene abundantly expressed in the upper third of pistils from opened and unopened flowers

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Abstract A polygalacturonase (PG) gene, *TPG7* (*Lyces;Pga1;8*), has been cloned from tomato (*Lycopersicon esculentum* Mill., cv. Rutgers). RNA blot analysis reveals that *TPG7* is highly expressed in pistils (ovary removed) from unopened and fully open flowers. Dissection of mature pistils demonstrated that *TPG7* expression is limited to the top third (stigmatic region) of the pistils. This is contrasted with another tomato PG, *TAPG4*, which is also expressed in the same region of the pistil but only in mature pistils from fully open flowers. Hybridization of the *TPG7* probe to anther RNA was nil to none and was barely detectable in RNA from leaf and flower abscission zones. The *TPG7* polypeptide shares 39% sequence identity with the tomato fruit PG and between 63% and 73% sequence identities with six other tomato PGs.

Key words Pistil · Polygalacturonase · Tomato · Gene

Introduction

Growth and development in higher plants often requires change in the cell wall structure and cell adhesion properties. Pectin comprises approximately 30% of the cell wall and a much greater proportion of the adhesive middle lamella between cells. In addition to pectin's structural importance to the cell wall, it has been suggested that it creates a matrix which prevents other hydrolases and transglycosylases having access to the hemicellulosic fraction of the cell wall (Carpita and Gibeau 1993). The hemicellulosic fraction is the cement that binds together the cellulose microfibrils in the cell wall. Change in the cell wall structure during growth therefore requires that the pectin matrix be first partially hydrolyzed to allow other enzymes access to the hemicellulosic matrix. The greatest constituent of pectins is polygalacturonic acid (Carpita and Gibeau 1993). Polygalacturonases (PGs) have been identified and characterized in ripening fruit, abscission, dehiscence, pollen maturation and rapidly expanding tissues (Hadfield and Bennett 1998).

In addition to a role for PGs in cell separation and cell enlargement, the oligogalacturonides released by endoPGs may have a role in developmental signaling and the activation of defense responses (Coté and Hahn 1994). Bellincampi et al. (1996) and Altamura et al. (1998) have suggested that oligogalacturonides may play a regulatory role in several auxin responses affecting growth and development. Moreover, oligogalacturonides released from the plant cell wall elicit an increase in pathogen-related (PR) gene expression (Coté and Hahn 1994).

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The EMBL, GenBank, and DDBJ accession number for the tomato polygalacturonase gene,*TPG7* (*Lyces;Pga1;8*), is AF072732

Materials and methods

Construction of a tomato genomic library

Tomato plants (*Lycopersicon esculentum* cv. Rutgers) were grown from seed under standard conditions in the greenhouse. Genomic DNA was isolated from young unexpanded leaves as described by Tai and Tanksley (1990). DNA was partially digested with *Sau3*A1 and size fractionated on a sucrose gradient (Ausubel et al. 1994). DNA fragments between 9 and 25 kbp were partially filled in using dGTP and dATP and ligated to λFix II arms (Stratagene, La Jolla, Calif., USA) that had been digested with *Xho*I and partially filled in using dTTP and dCTP. Recombi-

nant λDNA was packaged in vitro using a Gigapack Gold extract according to the manufacturer's instructions (Stratagene). *Escherichia coli* XL1-Blue MRA (F′) was infected with phage and approximately 4×10^5 plaque-forming units (pfu) were screened with a ^{32}P-nick translated *Bam*HI fragment from the pTAPG1 cDNA (Kalaitzis et al. 1995). Purification of positive clones, phage DNA preparation, restriction digestion and Southern blot hybridization were performed as described by Maniatis et al. (1982).

DNA sequencing and computer analysis

Restriction fragments of positive clones were subcloned into pT7/T3α18 (Gibco-BRL, Gaithersburg, Md., USA). To facilitate DNA sequencing, nested deletions of the subclones were prepared using exonuclease III and mung bean nuclease (Stratagene). DNA sequencing was performed by cycle sequencing using dye primer or dye terminator FS kit using the Applied Biosystem model 373 DNA sequencer (Perkin-Elmer Corp., Foster City, Calif., USA). The sequences were assembled and analyzed using the Genetics Computer Group (GCG) software package (Madison, Wis., USA).

RNA blot analysis

RNA was isolated as described in the protocol for the RNeasy Plant Mini Kit (Qiagen, Valencia, Calif., USA). Twenty micrograms of RNA were loaded per lane onto two identical gels, electrophoresed, blotted and each probed separately with ^{32}P-labeled *TPG7* and *TAPG4* probe. Hybridization conditions were 42 °C in 5× SSPE, 5× Denhardt's solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 60% formamide. Conditions used for the final wash of the blots were 0.2× SSC and 0.1% SDS at 55 °C.

Results and discussion

Identification and characterization of the *TPG7* gene

Recently, we cloned and characterized six tomato genes that are clustered into two separate groups in the tomato genome (Hong and Tucker 1998). The same tomato genomic library used to identify these six genes was screened again at a lower stringency (42 °C in 5× SSPE, 5× Denhardt's solution, 0.1% SDS, 100 μg/ml salmon sperm DNA, and 20% formamide) with a *TAPG1* probe (Hong and Tucker 1998). One genomic clone was selected for subcloning and sequencing. Sequence analysis revealed that the clone contained a PG gene. This gene was given the common name of *TPG7* and formal name of *Lyces;Pga1;8*. The coding sequence for *TPG7* was identified by comparison with *TAPG1*, *TAPG2*, and *TAPG4* cDNAs (Kalaitzis et al. 1995, 1997). *TPG7* consists of four exons interrupted by three introns of 68, 528, and 72 bp in length (Fig. 1A). The relative positions of the three introns are conserved between *TPG7* and the other tomato PGs (Hong and Tucker 1998).

The open reading frame of *TPG7* is 1194 bp long and encodes a polypeptide of 397 amino acids that includes a putative signal peptide cleaved after the alanine at position 26 (Von Heijne 1983). The number and positions of cysteine residues are highly conserved

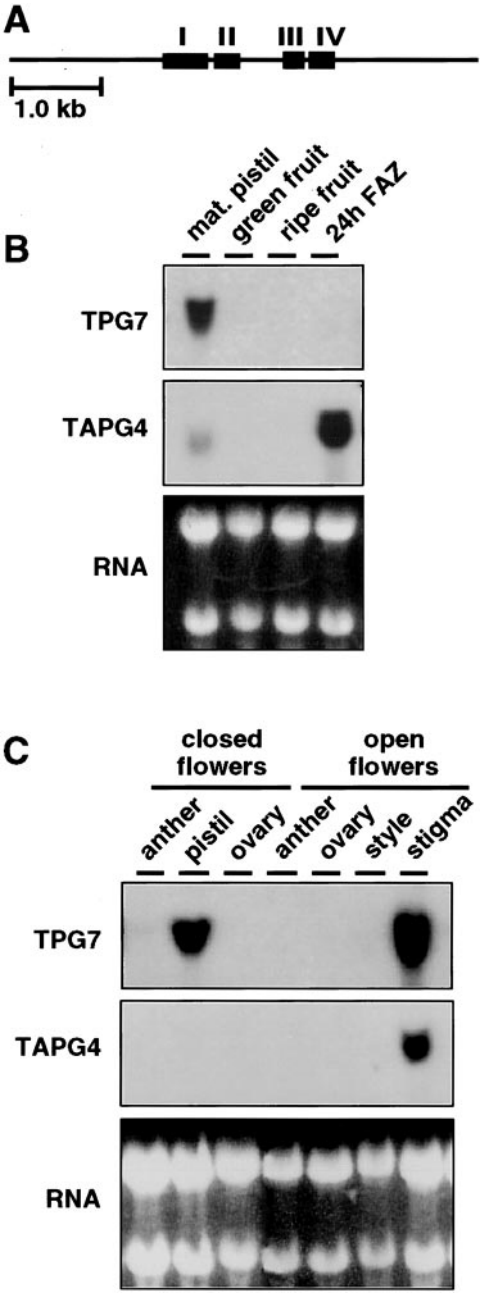

Fig. 1 *TPG7* gene structure (accession number AF072732) (**A**) and expression of *TPG7* transcript in different tissues (**B**, **C**). **A** The *boxes* indicate exons and the *solid line* the length of confirmed sequence. **B** Total RNA was isolated from pistils with ovaries removed from fully open flowers (*mat. pistil*), pericarp from green and red ripe fruit and flower pedicel abscission zones (*FAZ*) exposed to 25 μl/l ethylene for 24 h. **C** Total RNA was isolated from anthers, pistils (ovaries removed) and ovaries of nearly mature unopened flowers and fully open flowers. The pistils from open flowers were divided into stigmas (top third) and styles (bottom two thirds above the ovary) and ovaries. The same batch of RNA was used to create two identical blots and each probed separately with *TPG7* or *TAPG4* probe. Photos of the ethidium-bromide-stained gels are included to show relative loading of RNA into gels

among the tomato PGs, indicating a conserved tertiary structure.

Expression of *TPG7*

Sequence comparisons indicated that the first exon of *TPG7* shared the least sequence identity, less than 72%, with all other tomato PG genes so far identified. The first exon was amplified by polymerase chain reaction (PCR) and used as a probe for RNA blot analysis. *TPG7* transcript was found to accumulate most abundantly in mature pistils (Fig. 1B). No *TPG7* transcript was detected in mature green or ripe fruit (Fig. 1B), pistil styles, ovaries (Fig. 1C), stems, meristematic shoots, roots, or lateral root initials (data not shown). In some autoradiographs, a faint hybridization signal for *TPG7* probe was observed in RNA extracted from leaf and flower abscission zones and anthers (data not shown); however, reproducibility of the signal was poor and may be attributable to cross-hybridization to other PG transcripts.

The open reading frame of *TPG7* shares between 75% and 77% nucleotide sequence identity with *TAPG1*, *2*, and *4*. RNase protection demonstrated that *TAPG1*, *2* and *4* were expressed more abundantly in flower abscission zones than pistils (Kalaitzis et al. 1997). In Fig. 1, the tissue-specific accumulation of *TPG7* transcript is compared with that for *TAPG4* transcript. *TAPG4* transcript, as previously demonstrated accumulated most abundantly in flower abscission zones and to a lesser extent in mature pistils. Under the conditions used to produce the autoradiographs shown in Fig. 1, the *TPG7* probe did not cross-hybridize with *TAPG1*, *2* and *4* transcripts since no *TPG7* hybridization signal was observed in RNA extracted from flower abscission zones.

Expression of *TPG7* in pistils (ovary removed) occurs in both developing pistils of unopened flowers and fully mature pistils in open flowers (Fig. 1C). This contrasts with *TAPG4* which was detected only in mature pistils from open flowers (Fig. 1C). Mature, fully elongated pistils from open flowers were further divided into ovaries, styles and the upper one-third of the pistils. Expression of *TPG7* and *TAPG4* was limited to the upper third of mature pistils, which includes the stigma (Fig. 1C).

Similarity to other plant PGs

The GenBank database was searched for sequences similar to the *TPG7* open reading frame and the deduced polypeptides of all the plant PGs were compared and presented as a dendrogram (Fig. 2). The dendrogram graphically illustrates sequence similarities among the plant PGs and possible phylogenetic relationships. Seven of eight tomato PGs, including *TPG7* (63–73% amino acid sequence identity), grouped together along with one PG from peach (62% identity) and two PGs from melon (52% and 57% identity; (Fig. 2, group A). The tomato fruit PG (TOM6; 39% identity) grouped separate from the other tomato PGs (Fig. 2, group B). Based on their primary sequence, all of the PGs in groups A and B are endoPGs (Kalaitzis et al. 1997). There is no clear distinction between groups A and B for the tissue-specific expression of the included genes (Fig. 2). However, Hadfield and Bennett (1998) suggest that the proteins in group B may be distinguished by inclusion of an N-terminal peptide sequence that is post-translationally cleaved as demonstrated for the tomato fruit PG (DellaPenna and Bennett 1988). This is an interesting possibility that requires experimental verification. A third group of PGs, group C (Fig. 2), includes the exoPGs which are most notable for their expression in pollen (Tebbutt et al. 1994).

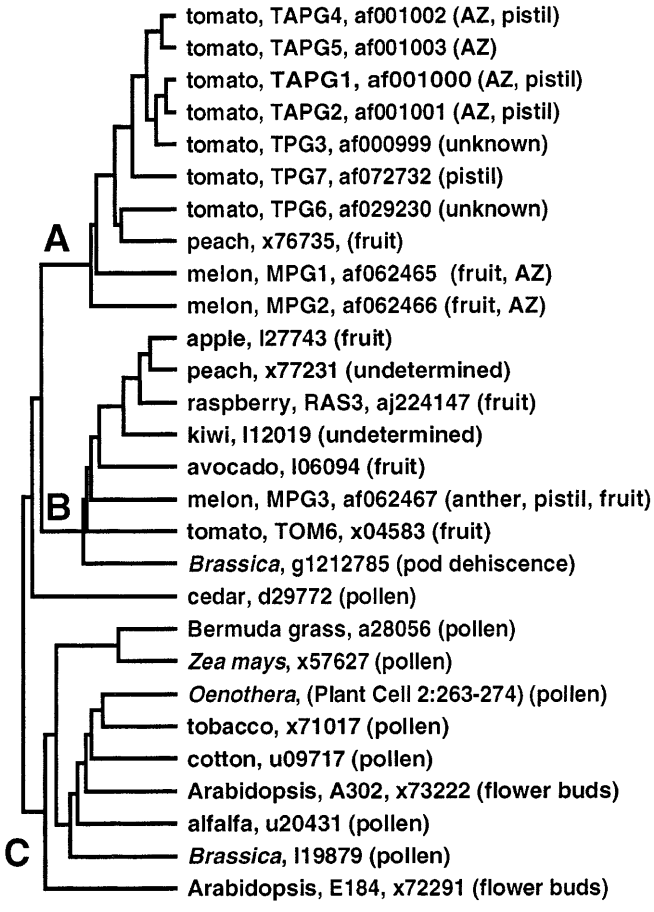

Fig. 2 Dendrogram prepared from the comparison of deduced amino acid sequences for plant PG cDNA and genomic sequences. Information listed for each PG includes the plant source, common name for selected clones, accession number or reference and published tissue expression patterns listed in *parentheses* from higher to lower abundance. *AZ* Abscission zones

The previously characterized abscission-expressed PGs, *TAPG1*, *2*, *4* and *5*, share significant sequence similarity in the first 300 bp of their 5′ upstream

sequences (Hong and Tucker 1998). Despite relatively high sequence identity between *TPG7* and the four *TAPG* open reading frames (Fig. 2), the 5′ upstream sequences of *TPG7* and the *TAPG*s do not share highly conserved sequence similarities. Consistent with this observation are distinct differences in the expression patterns for *TPG7* and the other four tomato PGs.

Concluding remarks

Although the specific function of the *TPG7* protein is not known, the abundance of *TPG7* transcript in pistils indicates that it may play a role in pistil development or pollination. RNase protection assays demonstrated that *TAPG1*, *2* and 4 were expressed in mature pistils in addition to being expressed in abscission zones (Kalaitzis et al. 1997). In addition to these PGs, at least two endo-1,4-β-glucanase (EGase) clones, TAC1 (Kalaitzis et al. 1999) and TPP18 (Milligan and Gasser 1995) have been demonstrated to be highly expressed in pistils. TPP18 (*cel4*) is most abundantly expressed in immature pistils (Milligan and Gasser 1995) and also rapidly expanding cells from leaves and hypocotyls (Brummell et al. 1997). PGs and EGases may work together to loosen cell walls in the mature pistil to facilitate pollen tube growth through the transmission track. In addition to loosening cells, oligogalacturonides released by PG activity may play a role in directing pollen tube growth and stimulation of a PR response in the vulnerable stigma tissue (Coté and Hahn 1994).

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